Chapter 5

Systemic Regulation of Root Nodule Formation

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1. Introduction

Most of legume plants produce *de novo* root lateral organs, root nodules, to accommodate their symbiotic bacteria, collectively called as rhizobia. Production of the *de novo* root organ is initiated by infection of rhizobia on surfaces of plant root tissues [1]. Rhizobia enter host tissues by two major strategies. One is the crack entry from the root epidermis where lateral roots emerge, and the other is the root hair-mediated invasion. Model legume plants, *Lotus japonicus* and *Medicago truncatula,* have adopted the latter mode of the infection pathway. In either case, infected rhizobia invade into host tissues through infection threads, which are tubular paths developed by invagination of the host plasma membrane. Concomitantly with the progression of infection processes from the epidermis into the cortex, a fraction of cortical cells beneath the site of the infection begin to divide and form a root nodule primordium. Invaded bacteria are released into cortical cells in the nodule primordium by an endocytic-like process, and differentiate into bacteroides. Whereas plants usually cannot utilize atmospheric nitrogen as a nutrient, bacteroides convert it to ammonium that is a usable nitrogen source for host plants. Host plants, on the other hand, provide the rhizobia with an energy source from photoassimilates, and compensate components that rhizobia lack for nitrogen fixation [2]. This symbiotic relationship enables legumes to grow under conditions where nitrogen sources are limited.

The nodule symbiosis with rhizobia is a unique feature of legumes among land plants. The infection processes are regulated by interaction with rhizobia. Nodulation factors (Nod factors) that are lipochitin oligosaccharides secreted by rhizobia trigger the symbiotic responses [3,4]. LysM containing receptor proteins, NFR1 and NFR5 in *L. japonicus,* and NFP and HCL in *M. truncatula* are involved in perception of Nod factors [5-9]. Direct binding of Nod factors to NFR1 and NFR5 was recently demonstrated [10]. Nod factor perception leads to activation of transcriptional networks involved in establishing the nodule symbiosis. This process depends on common *SYM* factors, which are required for both the nodule symbiosis and mycorrhizal

symbiosis [11-14]. While the mycorrhizal symbiosis is seen in the majority of land plant species, and has been thought to be evolved 400 million years ago, molecular clock data suggest that legumes has acquired the nodule symbiosis about 60 million years ago. Common *SYM*s are involved in transmission of nodulation signals into nuclei. Among common *SYM* factors, CCaMK (for calcium- and calmodulin-depednet protein kinase)/DMI3 plays pivotal roles in regulation of nodulation processes [15-17]. CCaMK localizes to nuclei, and interacts with its phosphorylation substrate, CYCLOPS/IPD3 [14,18,19]. gof-CCaMK spontaneously induces root nodules without rhizobial infection [16,17], indicating this protein substitutes for Nod factor signals in the cortical response for the root nodule organogenesis. Activation of CCaMK is sufficient for onset of gene expression required for the root nodule development. Thus, legumes have invented the nodule symbiosis by recruiting factors from the ancestral mycorrhizal symbiosis, and further have developed nodulation specific factors to acquire the nitrogen-fixing activity.

Although the root nodule symbiosis is beneficial to host plants, excessive nodulation interferes with plant growth, probably, due to a high energy cost against the nitrogen fixation. Therefore, legumes have developed negative feedback pathways that optimize total nodule number and mass in a single plant. A major pathway that regulates nodule number is known as autoregulation of nodulation (AON) [20,21], which is activated by nodulation, and systemically prevents subsequent formation of nodules through root-shoot communications. This systemic effect has been demonstrated by split root experiments. Infection with rhizobia to one part of the root results in reduction in the nodule number in the other part of the root that was inoculated 3-4 days after the first inoculation procedure [20,22]. Reciprocal grafting experiments with mutants defective in AON have further shown that the inhibitory effect seen in the root is mediated by the shoot [23-27]. Two types of long-distance signals that are derived from either the root or shoot have been postulated in AON to explain the root-shoot communication; The root-derived signal that was generated by early nodulation signaling is translocated to the shoot, and activates shoot-acting AON factors to produce the shoot-derived inhibitor (SDI), which, in turn, is transported down to the root, and inhibits nodulation through root-acting AON factors.

In the past decade, many mutants exhibiting impaired nodulation have been isolated, and genes responsible for symbiotic phenotypes have been identified. The accumulating lines of evidence led to basic models for the nodulation signaling pathway. Factors involved in AON have been identified from several legume species. Our knowledge of AON has greatly advanced at molecular levels by efforts of many researchers, although many pieces have remained to understand how AON systemically regulates nodulation. In this review, we highlight past work in this area and describe recent results obtained with forward and reverse genetic approaches as well as with biochemical works, along the basic scheme of AON that has been depicted by pioneer works.

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2. Shoot factors involved in AON

Genetic screens have identified loci that affect AON in several legume species. These mutants often display excessive nodules within an enhanced nodulation zone in either the presence of or the absence of nitrate, which possesses inhibitory effects on nodulation (see below). This phenotype is termed hypernodulation or supernodulation [18]. Soybean *nts*, *L. japonicus har1*, *M. truncatula sunn*, and pea *sym29* mutants exhibit the typical hypernodulation phenotype [24,27-29]. Grafting experiments have shown that shoots of these mutants confer excessive nodulation on wild type rootstocks, while mutant rootstocks that were grafted to the wild type scion do not exhibit the hypernodulation phenotype [23-27]. These results indicated that the shoot genotype determined the phenotype in the root, and genetically demonstrated that the root-shoot communication is important for the systemic and negative feedback regulation of the nodule formation.

Positional cloning of genes responsible for the hypernodulation phenotype revealed that AON has diverted factors involved in the shoot apical meristem homeostasis as shoot-acting factors. The causative genes of these mutants encode leucine-rich repeat (LRR) containing receptor protein kinases orthologous to each other [25,26,30,31]. It is expected that ligand-receptor interaction in the shoot regulates nodulation in the root. *In vitro* experiments have shown that the kinase domain of GmNARK/NTS possesses a transphosphorylation activity [32]. This kinase activity is abolished by amino acid substitutions at residues corresponding to sites of missense mutations that were found in loss-of-function *nts* alleles. The kinase activity is required for the GmNARK function, and phosphorylation of its substrates probably leads to the production of the SDI in the shoot. The GmNARK kinase domain phosphorylates two KAPPs (for kinase-associated protein phosphatases) [32]. The phosphorylated KAPPs dephosphorylated the autophosphorylated GmNARK kinase domain. The LRR receptor protein kinases are closest to Arabidopsis *CLV1* and rice *FON1*, which play a central role in the shoot apical meristem homeostasis, and restrict meristem sizes [33-35]. Multiple receptor proteins are involved in maintenance of meristem sizes in Arabidopsis [36]. Pea and *L. japonicus* genes orthologous to Arabidopsis *CLV2*, which encodes a LRR-containing transmembrane protein that interacts with and stabilizes CLV1 [37], are also involved in AON [38]. Furthermore, *L. japonicus* KLV that acts together with HAR1 is an LRR-receptor protein kinase with a high similarity to Arabidopsis RPK2/TOAD2 [39,40], which regulates the shoot meristem homeostasis in parallel with CLV1 [41]. These leguminous receptor proteins probably function as multiple complexes as it is for Arabidopsis counterparts [36,41]. HAR1, indeed, interacts with KLV in a transient expression system using *Nicotiana benthamiana* leaves [40]. Although the function of these leguminous genes in the shoot apical meristem has remained to be analyzed in detail, fasciated stems have been observed in Arabidopsis *klv* mutants and loss-of-function plants of *L. japonicus* and pea *CLV2* genes [38-40]. This phenotypic trait is often observed in mutants, such as *clv* mutants, where the negative feedback system to maintain meristem sizes is abortive and the shoot apical meristem is enlarged [33]. Besides stem fasciation, *klv* mutants further show disconnected vasculatures, indicating defect in cell differentiation or production of stem cells [39]. These observations imply that AON and the shoot meristem-associated pathway may utilize common or similar molecular strategies to maintain either the meristem

homeostasis or the nodule number. Alternatively, legumes may have developed pathways that specifically regulate nodule number in the root downstream of the shoot-acting receptor protein kinases.

3. Root-derived CLE peptides

In the shoot meristem regulation, CLV1 restricts meristem sizes through interaction with CLV3 that is a member of CLE (CLV3/ESR-related) small secreted peptide family [42-44]. CLV3 peptide is produced from its primary translational product by proteolytic processing [45]. By analogy to the CLV1-CLV3 pathway, the shoot-acting AON receptor protein kinases were expected to recognize CLE peptides that might act as the root-derived signal. Okamoto et al. have comprehensively analyzed expression of genes that encode CLE-peptide precursors in *L. japonicus*, and found that two *CLE* genes, referred to as *CLE-RS1* and *CLE-RS2*, specifically expressed in roots in response to rhizobial infection [46]. Similar approaches have also been performed in other leguminous species to identify *CLE* genes associated with the nodule development [47-50]. *MtCLE12* and *MtCLE13* from *M. truncatula* and *GmRIC1*, *GmRIC2*, and *GmNIC1* from soybean as well as *L. japonicus CLE-RS1* and *CLE-RS2* have activities to suppress the nodule formation [46,47,49,50]. Ectopic expression of these genes, except *GmNIC1*, in a part of the root system results in systemic inhibition of nodulation in the roots that have not ectopically expressed the *CLE* gene. This effect on nodulation depends on *HAR1* and *KLV* in *L. japonicus* and the corresponding shoot-acting AON genes in *M. truncatula* and soybean [40, 46,48,49,51]. These results are compatible with the idea that these *CLE* gene products are the root-derived signals. However, synthetic CLE peptides corresponding to the putative CLE-RS1, CLE-RS2, MtCLE12, and MtCLE13 failed to suppress nodulation [46,47].

Okamoto et al. have determined the structure of the mature CLE-RS2 [52]. This peptide is composed of 13 amino-acid residues corresponding to the conserved C-terminal CLE domain of its precursor, and the seventh proline residue is hydroxylated, and further posttranslationally modified with three residues of arabinose. This proline residue is conserved in all putative CLE peptides that are encoded by genes whose ectopic expression suppresses nodulation. The same arabinosylation has been also found at the seventh hydroxyproline residue of the CLV3 peptide. This modified CLV3 peptide interacts more strongly with the ectodomain of CLV1 [53]. The synthetic arabinosylated CLE-RS1 and CLE-RS2 bound to HAR1 protein, but not to a loss-of-function HAR1 derivative with an amino acid substitution at a residue critical for ligand binding of the CLV1 family receptor kinases in Arabidopsis [52,54]. The activity of the CLE-RS1 and CLE-RS2 peptides to suppress nodulation has been demonstrated by feeding experiments with the synthetic arabinosylated peptides [52]. The synthetic CLE-RS2 and CLE-RS1 peptides that were fed from cotyledon surfaces suppressed nodulation depending on *HAR1,* but those without arabinosylation failed. The arabinosylation is essential for binding to HAR1 and the activity to inhibit nodulation. These analyses have shown that the arabinosylated CLE-RS2 and CLE-RS1 are ligands for HAR1.

Unlike the short-distance communication between CLV1 and CLV3 (these genes express in restricted region of the shoot apical meristem), long-ranged transport is necessary for the root-

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derived CLE peptides to interact with HAR1. The xylem often mediates the transport of molecules from the root to the shoot [55]. The arabinosylated CLE-RS2 was detected in xylem sap collected from soybean shoots whose roots have been transformed to express *CLE-RS2* [52]. The secreted mature peptide is thought to be transported to the shoot in the xylem. This is consistent with spatial expression patterns of *HAR1, KLV*, and *NTS*/*GmNARK*; They express in vascular tissues [40,56]. The arabinosylated CLE-RS2 and CLE-RS1 peptides that were transported in the xylem are thought to bind with HAR1, and exert the inhibitory effect on nodulation.

4. Expression of *CLE* genes that encode the root-derived signal

Transcriptional regulation of the root-derived signal is an important step to regulate AON. The expression in response to rhizobial infection is mediated by the early signaling pathway required for nodulation processes [47]. Transcription factors that are involved in the early nodulation signaling, such as NSP1, NSP2, and NIN, are required for expression of *MtCLE12* and *MtCLE13* in response to rhizobial infection. These transcription factors are essential for formation of the nodule primordium, which is initiated by division of cortical cells beneath epidermal cells infected by rhizobia [57-60]. It has been shown that ectopic expression of *NIN* induces cortical cell division without rhizobial infection [61]. Mortier et al. have shown detailed spatial expression patterns of *MtCLE12* and *MtCLE13* during the course of the nodule meristem formation [47]. These genes begin to express in cortical cells beneath infection sites in the epidermis [47]. The expression is sustained in dividing cortical cells in the incipient nodule primordium, and is restricted in the apical zone of elongated indeterminate type nodules. The apical region corresponds to the meristematic and early infection zones. Expression patterns similar to those of *MtCLE* genes have also shown in the case of *GmRIC2* [49]. In the current model of the nodule meristem formation, perception of Nod factors that are secreted by rhizobia triggers the nodule meristem formation through activation of cytokinin signaling [62-65]. Cytokinin induces expression of *NSP1, NSP2,* and *NIN* [62-64,66], although *NSP2* expression is transient and repressed within 3 hours after the treatment [67]. *MtCLE13* expression in the root treated with exogenous cytokinin is detected in inner cortical cells [68], which is consistent with the site where where *MtCLE13* begins to express when roots were inoculated with rhizobia. These observations indicate that *CLE* gene expression is associated with the nodule primordium or meristem formation.

In addition to rhizobial infection, expression of *CLE-RS2* and *GmNIC1* is induced by nitrate, which also has an inhibitory effect on nodulation [46,50]. *nts* mutants were originally identified as nitrate-tolerant symbiotic mutants [28]. *har1* and *klv* mutants also exhibit the nitrate-tolerance [39]. It is likely that the different inputs, rhizobial infection and nitrate, activate the same *CLE* gene in *L. japonicus,* and interfere with nodulation through AON. It has been shown that NIN-like proteins (NLPs) targets nitrate-responsive elements (NREs) that were found in promoters of nitrate-inducible genes, such as *NIR1*, and activate the gene expression in Arabidopsis [69-71]. All 9 Arabidopsis NLPs are able to bind to NREs and possess potentials to activate gene expression in transient expression assay and *in vitro*

experiments. NIN has been thought to be derived from a member of NLPs [72,73]. All of domains that are conserved in NLPs are present in the NIN protein except the GAF-related domain in the N-terminal region, which is necessary for NLPs to sense a nitrate-signal that post-translationally activates the NLP transcriptional activity [70]. NIN-binding nucleotide sequences that were found in promoters of NIN-target genes are similar to those of NREs [61]. NIN and NLPs may target *CLE* genes whose mature translational products act as the root-derived AON signals, and activate the gene expression in response to different stimuli, rhizobial infection and the nitrate-supply, respectively. There may be an evolutionary link between pathways for nitrate-responses and AON. Nitrate also systemically regulates root architecture in Arabidopsis [74,75].

5. Shoot-derived inhibitors

The SDI is thought to be produced depending on activation of the shoot-acting receptor protein kinases. Expression of *GmNARK* in the phloem has suggested that the SDI is produced in the vascular tissue and transported in the phloem. Lin et al. have shown the presence of the SDI activity in leaf extracts from soybean [76]. They developed a feeding system from petioles, and found the activity that suppresses nodulation in aqueous extracts prepared from leaves of inoculated wild type soybean. This activity is generated depending on Nod factors and *GmNARK,* and effective in both wild type plants and *nts* mutants. The generality of the activity was demonstrated with leaf extracts from inoculated *M. truncatula*. These properties of the activity are consistent with that of the presumptive SDI. The factor with the activity is a heat-stable small molecule of < 1 kDa and resistant against either RNase or Proteinase K. It is likely that SDI is neither a protein nor an RNA.

Although the shoot-derived molecule that acts as the SDI has not yet been identified, several phytohormones have shown inhibitory effects on nodulation [77] and associated with AON. Auxin has been postulated to be involved in nodulation [78], and is transported from the shoot to the root by cell-to-cell transport mediated by auxin carriers, and also thought to travel in the phloem. *M. truncatula SUNN* is involved in regulation of this long-distance auxin transport. van Noorden et al. have shown that auxin transport from the shoot to the root is downregulated by inoculation with rhizobia in wild type plants, whereas the rate of the transport is not changed in the *sunn* mutant, and auxin is more abundant in the mutant root compared to the wild type [79]. They have proposed a model of auxin action in AON based on their observations; Downregulation of the long-distance transport of auxin by AON leads to reduction in the auxin level in the roots, resulting in decreased efficiencies of the nodule initiation. Indeed, local application of an auxin transport inhibitor, N-(1-naphthyl)phthalamic acid, to a hypocotyl results in reduction of nodule number in the *sunn* mutant, but not in the wild type plant [79]. Unlike cytokinin, involvement of auxin in the nodule primordium formation has remained obscure. It would be important to elucidate roles of auxin in the root nodule development for understanding relation with AON.

In soybean, on the other hand, it has been reported that metabolic pathways of jasmonic acid in the shoot are altered by *nts* mutations. Kinkema et al. have found that transcripts of genes

encoding key enzymes controlling jasmonic acid biosynthesis and of jasmonic acid-responsive genes are accumulated in the shoot of *nts* mutants compared to wild type plants [80]. They have also shown that foliar application of a jasmonic acid biosynthesis inhibitor reduces nodule number. These results suggest that jasmonic acid is a negative regulator in AON. However, other two groups have shown that foliar applied methyl jasmonic acid downregulates nodulation in soybean and *L. japonicus* [81,82]. Involvement of jasmonic acid or methyl jasmonic acid in AON remains controversial. It has been also reported that foliar application of brassinosteroides to loss-of-function *GmNARK* plants suppresses nodule formation [83]. Grafting experiments have shown that shoots of a pea mutant of which causative gene encodes a brassinosteroides biosynthesis enzyme causes decrease in nodule number in wild type rootstacks [84]. It would be required for further analysis on action of brassinosteroides in systemic inhibitory effects.

6. Root-acting factors involved in inhibition of nodulation

According to the scenario described above, the mature CLE peptides whose genes express in response to rhizobial infection act as the root-derived signals, and activate shoot-acting receptor protein kinases including HAR1 and KLV to generate the SDI, which in turn inhibits nodulation. Root-acting AON factors involved in production of the root-derived signal or perception of the SDI are required for AON to exert the inhibitory effect. Root-specific hypernodulation mutants, pea *nod3*, *L. japonicus plenty,* and *tml*/*rdh1*, and *M. truncatula rdn1* and *sickle* have been isolated [85-90]. Reciprocal grafting experiments have shown that root genotypes of these genetic loci influence nodulation.

PLENTY seems to work in a pathway different form theHAR1-mediated AON, since grafting of a *har1* scion resulted in an additive phenotype with respect to number of the root nodules [88]. It has been implied that nodule number is regulated by multiple pathways. Ethylene-insensitive *sickle* mutants have shown that ethylene signaling influence nodule number [91]. The causative gene of this mutant encodes an ortholog of Arabidopsis EIN2 that is involved in ethylene signaling [90]. Similar to *sunn* mutants, downregulation of the long-distance auxin transport in response to rhizobial infection dose not occur in the *sickle* mutant [92]. However, *SICKLE* seems to regulate the nodule number independently of the *SUNN*-mediated pathway, because *sickle sunn* double mutants exhibited a novel hypernodulation phenotype [27]. Nodules densely cover the whole root length in *sunn* mutants, while nodules are formed at the limited region corresponding to the first inoculation zone in *sickle* mutants [91]. Auxin transport inhibition may not be unique to AON.

Pea *NOD3* and *MtRDN1* are orthologous genes, and encode a protein of the endosomal system with unknown function [89]. Similar to *sunn* mutants, *rdn1* mutants show the hypernodulation phenotype that was moderately suppressed by nitrate, and the shorter root phenotype. *RisfixC* locus allelic to *nod3* exhibits an interesting phenotype. A wild type scion that was grafted to a *RisfixC* rootstock produced adventitious roots with the hypernodulation phenotype [93]. Approach-grafting experiments (two plants with intact roots are grafted at stems) have shown

that the first inoculation of the *nod3* mutant root fails to suppress nodulation in the wild type root that were inoculated 7 days after the first inoculation, resulting in increase in nodule number compared to the wild type control root [94]. These results suggested that *nod3* mutations systemically affected nodulation. Schnabel et al. have shown that a *GUS* reporter construct for the *MtRND1* promoter intensely expresses in the vascular tissues, in particular, the area of the xylem, throughout the root [89]. NOD3/MtRND1 may be involved in early events of the AON pathway associated with production or transportation of the root-derived signal.

Contrary to NOD3/MtRND1, *L. japonicus* TML is a root-acting AON factor that functions after production of the SDI. *L. japonicus tml* and *rdh1* are allelic mutants [95]. TML has been shown to function downstream of the root-derived CLE peptides [95]. The systemic inhibition of the nodulation by either *CLE-RS1* or *CLE-RS2* is suppressed by *tml* mutations, similar to *har1* and *klv* mutations. Unlike the *plenty* mutant, shoot scions of *har1* and *klv* mutants do not enhance the hypernodulation phenotype of *tml* rootstocks [87,95]. TML is thought to work in the same genetic pathway with HAR1 and KLV. Furthermore, the inverted-Y grafting has shown that a *tml* root that was inserted into a hypocotyl of a wild type seedling with the intact root does not affect efficiencies of nodulation in the wild type root [87]. This result suggested that the *tml* mutation does not systemically affect nodulation, and the *tml* root is insensitive to the SDI.

TML encodes a kelch repeat-containing F-box protein [95]. F-box proteins are subunits of SCF E3 ubiquitin ligase complexes that lead to inactivation of substrate proteins through ubiquitination and subsequent degradation by 26S proteasome. F-box proteins have been thought to confer substrate specificities on SCF E3 ubiquitin ligase complexes. TML protein that was fused to GFP localizes into nuclei. This subcellular localization implies that TML targets transcription factors, similarly to Arabidopsis kelch-repeat F-box protein, FKF1 [96]. Temporal and spatial expression patterns of a GUS reporter construct for the *TML* promoter has suggested that TML suppresses development of the root nodule meristem after initiation of cortical cell division [95]. In addition to the nodule primordium, *TML* constitutively expresses in the root apex transition zone, which is at developmental stages earlier than the susceptible region for rhizobial infection. *tml* mutants produce excess infection threads in the epidermis. One hypothesis is that TML indirectly inhibits formation of infection threads prior to rhizobial infection at the root transition zone. TML may exert inhibition of nodulation with two modes of actions at the site of rhizobial infection and of the nodule meristem formation.

7. Which steps of nodulation are influenced by AON?

Although molecular mechanisms, by which nodule formation is suppressed in the root, have been largely unknown, several results may be able to speculate how AON influences nodulation. Rhizobia usually infect at the root tip region where elongation of root hairs occurs, and nodule formation is initiated at the infection site. Then, nodule formation at the root region that is developmentally younger than the first inoculated region is suppressed. Thereby, nodule formation is limited at the first inoculation zone of the root. The mutants exhibiting

impaired AON show increases in number of both infection threads and nodules. Nodule formation in these mutants is deregulated with respect of the nodule density and the region that form nodules. It is suggested that AON influences on early stages of rhizobial infection and local regulation of nodule number. Identification of loss-of-function mutants of a *L. japonicus* cytokinin receptor gene (*LHK1*) as a suppressor of the *har1* hypernodulation phenotype has suggested that AON may act downstream of LHK1 in the nodule initiation [62]. Spatial expression patterns of *TML* suggest that AON targets early nodulation signaling pathway required for rhizobial infection, and inhibits cell division in the cortex [87,95].

Ectopic *MtCLE12* and *MtCLE13* abolished expression of *MtENOD11* expression in the root epidermal cells in response to rhizobial infection [47]. Although *MtENOD11* is unknown function, NSP1-NSP2 transcription factor complex and ERF/AP2 family transcription factors, ERNs, target the *MtENOD11* promoter and activate the gene expression [97-99]. The suppression of *MtENOD11* expression suggests that one of sites AON targets is the early nodulation signaling pathway, including NSP1 and NSP2. It is compatible with expression of *TML* in the root apex transition zone. Murakami et al. have shown that *NSP2* expression is remarkably downregulated at the root tip region within 1 day after rhizobial infection [100]. *MtENOD11* is activated by Nod factor treatment. The expression in the root tip is repressed within 1 day after the treatment, which is consistent with downregulation of *NSP2* expression. Interestingly, this *MtENOD11* repression spatially and temporally regulated is suppressed in *nin* mutants [101]. NIN is required for expression of *MtCLE12* and *MtCLE13,* and NSP2 is required for *NIN* expression in response to rhizobial infection. There may be the negative feedback regulation mediated by *NSP2* and *NIN*. However, it is unlikely that the repression of *NSP2* is mediated by the root-shoot communication of AON, because split-root experiments have shown that it takes 3 days to systemically suppress nodulation in *L. japonicus* [102]. In soybean, on the other hand, *GmNIC1* overexpression locally, but not systemically, suppresses nodulation [50]. This effect of the overexpression depends on GmNARK. Some aspects of local inhibitory effects in soybean may be mediated by LRR-receptor protein kinases.

Saur et al. have shown a strong reduction of *NIN* expression in roots overexpressing *MtCLE12* when compared with controls at 21 days after inoculation [103]. They have proposed a negative feedback regulation that implicates cytokinin signaling and NIN in AON. Type-A response regulators highly express in roots where *MtCLE12* overexpresses at the same time point, although there are no significant differences at 8 days after inoculation. Type-A response regulators are responsible for the negative-feedback regulation of cytokinin signaling [104], and may repress cytokinin-dependent *NIN* expression. Arrested primordia that were composed of cells that originate from the cortex, endodermis, and pericycle were observed in roots overexpressing MtRR9 type-A response regulator [105].

In the shoot apical meristem homeostasis, the CLV1-CLV3 pathway represses a gene encoding a homeobox transcription factor, WUSCHEL, which directly activates *CLV3* gene and represses expression of type-A response regulator genes [106,107]. *WUSCHEL*, itself, is activated by cytokinin. This transcription factor plays a critical role in the feedback loop to maintain the homeostasis of the shoot apical meristem. A WUSCHEL-related homeobox transcription factor, WOX5, expresses in the nodule primordium of *M. truncatula,* [108]. Although a role of

MtWOX5 in the nodule development has not yet been clarified, induction of this gene in response to treatment with auxin, but not cytokinin, suggests that auxin is required for expression of genes that may act in regulation of cortical cell division. An auxin-responsive synthetic promoter, *DR5,* is often used for monitoring distribution of auxin [109,110]. Intense expression of GFP protein whose expression is under the control of the *DR5* promoter is detected in cortical cells beneath the infection site in the epidermis, and sustained in developing nodules [111]. The expression is attenuated in *L. japonicus* roots overexpressing *CLE-RS2*. On the other hand, in *har1* background, the *DR5* construct expresses in broad area of the infected root, suggesting that auxin is more abundantly accumulated in the mutant root. It is consistent with the result obtained by direct measurement of auxin levels in *sunn* mutants [79]. Auxin activates cell division of pericycle cells that is origin of lateral root primordia. Excessive cell division in the root pericycle as well as cortical cell division has been observed in the *har1* mutant [29]. This alteration of the *DR5* expression suggests that AON influences auxin distribution at the site of the nodule initiation and development, leading to inhibition of cell division. In this context, auxin is a positive regulator of the nodule formation. Auxin transport inhibitors elicit pesudonodule formation in *M. sativa* and *M. truncatula* [112,113], indicating alteration of auxin distribution is an important cue for the nodule formation as well as cytokinin.

8. Conclusion

Forward genetic analyses have greatly contributed to identify novel factors that are involved in AON. The series of investigations revealed that AON has diverted factors required for the homeostasis of the shoot apical meristem as the shoot-acting factors. The ligand-receptor relationship between HAR1 and the root-derived CLE peptides has been clearly demonstrated by the reverse genetic approaches and biochemical assays. The similarity of AON to the CLV1-CLV3 pathway suggests that AON may have recruited functions from pathways that generally work in non-leguminous species. How legumes have been molecularly specialized from non-leguminous species is an important question to understand molecular basis on the root nodule symbiosis. Studies on AON would shed light on this fundamental question besides elucidation of the molecular nature of the systemic inhibitory pathway. There remains three major questions to to elucidate AON at molecular levels: (1) How expression of the root-derived signals is regulated, (2) What is a bona-field SDI, (3) Molecular mechanisms, by which nodulation is suppressed in the root. Lin et al. have shown the SDI activity in aqueous extracts prepared from leaves of inoculated wild type soybean. Forward genetics has identified TML as the root-acting AON factor involved in suppression of nodulation downstream of the SDI. These findings as well as data obtained by efforts of many researchers would be clues to resolve these questions.

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References

[1] Oldroyd GE, Downie JA. Coordinating nodule morphogenesis with rhizobial infection in legumes. Annual Review of Plant Biology 2008;59 519-546.

[2] Hakoyama T, Niimi K, Watanabe H, Tabata R, Matsubara J, Sato S, Nakamura Y, Tabata S, Jichun L, Matsumoto T, Tatsumi K, Nomura M, Tajima S, Ishizaka M, Yano K, Imaizumi-Anraku H, Kawaguchi M, Kouchi H, Suganuma N. Host plant genome overcomes the lack of a bacterial gene for symbiotic nitrogen fixation. Nature 2009;462(7272) 514-517.

[3] Ehrhardt DW, Wais R, Long SR. Calcium spiking in plant root hairs responding to Rhizobium nodulation signals. Cell 1996;85(5) 673-681.

[4] Esseling JJ, Lhuissier FGP, Emons AMC. Nod factor-induced root hair curling: continuous polar growth towards the point of Nod factor application. Plant Physiolgy 2003;132(4) 1982-1988.

[5] Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J. A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. Nature 2003;425(6958) 637-640.

[6] Radutoiu S, Madsen LH, Madsen EB, Felle HH, Umehara Y, Grønlund M, Sato S, Nakamura Y, Tabata S, Sandal N, Stougaard J. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature 2003;425(6958):585-592.

[7] Limpens E, Franken C, Smit P, Willemse J, Bisseling T, Geurts R. LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science 2003;302(5645):630-633.

[8] Arrighi JF, Barre A, Ben Amor B, Bersoult A, Soriano LC, Mirabella R, de Carvalho-Niebel F, Journet EP, Ghérardi M, Huguet T, Geurts R, Dénarié J, Rougé P, Gough C. The *Medicago truncatula* lysin motif-receptor-like kinase gene family includes *NFP* and new nodule-expressed genes. Plant Physiolgy 2006;142(1) 265–279.

[9] Smit P, Limpens E, Geurts R, Fedorova E, Dolgikh E, Gough C, Bisseling T. Medicago LYK3, an entry receptor in rhizobial nodulation factor signaling. Plant Physiolsy 2007;145(1):183-191.

[10] Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, Maolanon N, Vinther M, Lorentzen A, Madsen EB, Jensen KJ, Roepstorff P, Thirup S, Ronson CW, Thygesen MB, Stougaard J. Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. Proceedings of the National Academy of Sciences of the United States of America 2012;109(34):13859-13864.

[11] Kistner C, Parniske M. Evolution of signal transduction in intracellular symbiosis. Trends in Plant Science 2002;7(11) 511-518.

[12] Kaló P, Gleason C, Edwards A, Marsh J, Mitra RM, Hirsch S, Jakab J, Sims S, Long SR, Rogers J, Kiss GB, Downie JA, Oldroyd GE. Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 2005;308(5729)1786-1789.

[13] Heckmann AB, Lombardo F, Miwa H, Perry JA, Bunnewell S, Parniske M, Wang TL, Downie JA. *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. Plant Physiolgy 2006;142(4) 1739-1750.

[14] Messinese E, Mun JH, Yeun LH, Jayaraman D, Rougé P, Barre A, Lougnon G, Schornack S, Bono JJ, Cook DR, Ané JM. A novel nuclear protein interacts with the symbiotic DMI3 calcium- and calmodulin-dependent protein kinase of *Medicago truncatula*. Molecular Plant-Microbe Interactions 2007;20(8) 912-921.

[15] Catoira R, Galera C, de Billy F, Penmetsa RV, Journet EP, Maillet F, Rosenberg C, Cook D, Gough C, Dénarié J. Four genes of *Medicago truncatula* controlling components of a nod factor transduction pathway. Plant Cell 2000;12(9) 1647-1666.

[16] Gleason C, Chaudhuri C, Yang T, Muñoz A, Poovaiah BW, Oldroyd GED. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. Nature 2006;441(7097) 1149-1152.

[17] Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, Miwa H, Nakagawa T, Sandal N, Albrektsen AS, Kawaguchi M, Downie A, Sato S, Tabata S, Kouchi H, Parniske M, Kawasaki S, Stougaard J. Deregulation of a Ca^{2+} calmodulin-dependent kinase leads to spontaneous nodule development. Nature 2006;441(7097) 1153-1156.

[18] Yano K, Yoshida S, Müller J, Singh S, Banba M, Vickers K, Markmann K, White C, Schuller B, Sato S, Asamizu E, Tabata S, Murooka Y, Perry J, Wang TL, Kawaguchi M, Imaizumi-Anraku H, Hayashi M, Parniske M. CYCLOPS, a mediator of symbiotic intracellular accommodation. Proceedings of the National Academy of Sciences of the United States of America 2008;105(51) 20540-20545.

[19] Horváth B, Yeun LH, Domonkos A, Halász G, Gobbato E, Ayaydin F, Miró K, Hirsch S, Sun J, Tadege M, Ratet P, Mysore KS, Ané JM, Oldroyd GED, Kaló P. *Medicago truncatula IPD3* is a member of the common symbiotic signaling pathway required

for rhizobial and mycorrhizal symbioses. Molecular Plant-Microbe Interactions 201;24(11) 1345-1358.

[20] Kosslak, RM, Bohlool BB. Suppression of Nodule Development of One Side of a Split-Root System of Soybeans Caused by Prior Inoculation of the Other Side. Plant Physiology 1984;74(1) 125-130.

[21] Caetanoanolles G, Gresshoff P.M. Early Induction of Feedback Regulatory Responses Governing Nodulation in Soybeen. Plant Science 1990;71(1) 69-81.

[22] Olsson JE, Nakao P, Benbohlool B, Gresshoff PM. Lack of Systemic Suppression of Nodulation in Split Root Systems of Supernodulating Soybean (*Glycine max* [L.] Merr.) Mutants. Plant Physiology 1989;90(4) 1347-1352.

[23] Delves AC, Mathews A, Day DA, Carter AS, Carroll BJ, Gresshoff PM. Regulation of the Soybean-Rhizobium Nodule Symbiosis by Shoot and Root Factors. Plant Physiology 1986;82(2) 588-590.

[24] Sagan M, Duc G. *Sym28* and *Sym29*, two new genes involved in regulation of nodulation in pea (*Pisum sativum L*). Symbiosis 1996;20(3) 229-245.

[25] Krusell L, Madsen LH, Sato S, Aubert G, Genua A, Szczyglowski K, Duc G, Kaneko T, Tabata S, de Bruijn F, Pajuelo E, Sandal N, Stougaard J. Shoot control of root development and nodulation is mediated by a receptor-like kinase. Nature 2002;420(6914) 422-426.

[26] Nishimura R, Hayashi M, Wu GJ, Kouchi H, Imaizumi-Anraku H, Murakami Y, Kawasaki S, Akao S, Ohmori M, Nagasawa M, Harada, K, Kawaguchi M. HAR1 mediates systemic regulation of symbiotic organ development. Nature 2002;420(6914) 426-429.

[27] Penmetsa RV, Frugoli JA, Smith LS, Long SR, Cook DR. Dual genetic pathways controlling nodule number in *Medicago truncatula*. Plant Physiology 2003;131(3) 998-1008.

[28] Carroll BJ, McNeil DL, Gresshoff PM. Isolation and properties of soybean [*Glycine max* (L.) Merr.] mutants that nodulate in the presence of high nitrate concentrations. Proceedings of the National Academy of Sciences of the United States of America 1985;82(12) 4162-4166.

[29] Wopereis J, Pajuelo E, Dazzo FB, Jiang QY, Gresshoff PM, de Bruijn FJ, Stougaard J, Szczyglowski K. Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. Plant Journal 2000;23(1) 97-114.

[30] Arai M, Hayashi M, Takahashi M, Shimada S, Harada K. Expression and sequence analysis of systemic regulation gene for symbiosis, *NTS1*/*GmNARK* in supernodulating soybean cultivar, Sakukei 4. Breeding Science 2005;55(2) 147-152.

[31] Schnabel E, Journet EP, de Carvalho-Niebel F, Duc G, Frugoli J. The *Medicago truncatula SUNN* gene encodes a CLV1-like leucine-rich repeat receptor kinase that regulates nodule number and root length. Plant Molecular Biology 2005;58(6) 809–822.

[32] Miyahara A, Hirani TA, Oakes M, Kereszt A, Kobe B, Djordjevic MA, Gresshoff PM. Soybean Nodule Autoregulation Receptor Kinase Phosphorylates Two Kinase-associated Protein Phosphatases *in Vitro*. Journal of Biological Chemistry 2008;283(37) 25381–25391.

[33] Clark SE, Jacobsen SE, Levin JZ, Meyerowitz EM. The *CLAVATA* and *SHOOT MERISTEMLESS* loci competitively regulate meristem activity in *Arabidopsis*. Development 1996;122(5) 1567-1575.

[34] Clark SE, Williams RW, Meyerowitz EM. The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in *Arabidopsis*. Cell 1997;89(4) 575-585.

[35] Suzaki T, Sato M, Ashikari M, Miyoshi M, Nagato Y, Hirano HY. The gene *FLORAL ORGAN NUMBER1* regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1. Development 2004;131(22) 5649-5657.

[36] Perales M, Reddy GV. Stem cell maintenance in shoot apical meristems. Current Opinion in Plant Biology 2012;15(1) 10-16.

[37] Jeong S, Trotochaud AE, Clark SE. The *Arabidopsis CLAVATA2* gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. Plant Cell 1999;11(10) 1925-1933.

[38] Krusell L, Sato N, Fukuhara I, Koch BEV, Grossmann C, Okamoto S, Oka-Kira E, Otsubo Y, Aubert G,Nakagawa T, Sato S, Tabata S, Duc G, Parniske M, Wang TL, Kawaguchi M, Stougaard J. The *Clavata2* genes of pea and *Lotus japonicus* affect autoregulation of nodulation. Plant Journal 2011;65(6) 861-871.

[39] Oka-Kira E, Tateno K, Miura K, Haga T, Hayashi M, Harada K, Sato S, Tabata S, Shikazono N, Tanaka A, Watanabe Y, Fukuhara I, Nagata T, Kawaguchi M. *klavier* (*klv*), a novel hypernodulation mutant of *Lotus japonicus* affected in vascular tissue organization and floral induction. Plant Journal 2005;44(3) 505-515.

[40] Miyazawa H, Oka-Kira E, Sato N, Takahashi H, Wu GJ, Sato S, Hayashi M, Betsuyaku S, Nakazono M, Tabata S, Harada K, Sawa S, Fukuda H, Kawaguchi M. The receptor-like kinase KLAVIER mediates systemic regulation of nodulation and non-symbiotic shoot development in *Lotus japonicus*. Development 2010;137(24) 4317-4325.

[41] Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, Stahl Y, Simon R, Yamaguchi-Shinozaki K, Fukuda H, Sawa S. RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in *Arabidopsis*. Development 2010; 137(22):3911-3920.

[42] Fletcher LC, Brand U, Running MP, Simon R, Meyerowitz EM. Signaling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. Science 1999;283(5409) 1911-1914.

[43] Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R. Dependence of stem cell fate in *Arabidopsis* an a feedback loop regulated by CLV3 activity. Science 2000;289(5479) 617-619.

[44] Cock JM, McCormick S. A large family of genes that share homology with *CLAVATA3*. Plant Physiology 2001;126(3) 939-942.

[45] Matsubayashi Y. Post-Translational Modifications in Secreted Peptide Hormones in Plants. Plant and Cell Physiology 2011; 52(1) 5-13.

[46] Okamoto S, Ohnishi E, Sato S, Takahashi H, Nakazono M, Tabata S, Kawaguchi M. Nod factor/nitrate-induced *CLE* genes that drive HAR1-mediated systemic regulation of nodulation. Plant and Cell Physiology 2009;50(1) 67-77.

[47] Mortier V, Den Herder G, Whitford R, Van de Velde W, Rombauts S, D'Haeseleer K, Holsters M, Goormachtig S. CLE Peptides Control *Medicago truncatula* Nodulation Locally and Systemically. Plant Physiology 2010; 153(1) 222-237.

[48] Mortier V, Fenta BA, Martens C, Rombauts S, Holsters M, Kunert K, Goormachtig S. Search for nodulation-related *CLE* genes in the genome of *Glycine max*. Journal of Experimental Botany 2011;62(8) 2571-2583.

[49] Lim CW, Lee YW, Hwang CH. Soybean nodule-enhanced CLE peptides in roots act as signals in GmNARK-mediated nodulation suppression. Plant and Cell Physiology 2011; 52(9) 1613-1627.

[50] Reid DE, Ferguson BJ, Gresshoff PM. Inoculation- and Nitrate-Induced CLE Peptides of Soybean Control NARK-Dependent Nodule Formation. Molecular Plant-Microbe Interactions 2011;24(5) 606-618.

[51] Saur IM, Oakes M, Djordjevic MA, Imin N. Crosstalk between the nodulation signaling pathway and the autoregulation of nodulation in *Medicago truncatula*. New Phytologist 2011;190(4) 865–874.

[52] Okamoto S, Shinohara H, Mori T, Matsubayashi Y, Kawaguchi M. Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nature communications 2013;4:2191 | DOI: 10.1038/ncomms3191. http://www.nature.com/ncomms/2013/130812/ncomms3191/full/ncomms3191.html (accessed 7 Nov 2013)

[53] Ohyama K, Shinohara H, Ogawa-Ohnishi M, Matsubayashi Y. A glycopeptide regulating stem cell fate in *Arabidopsis* thaliana. Nature Chemical Biology 2009;5(8) 578-580.

[54] Shinohara H, Moriyama Y, Ohyama K, Matsubayashi Y. Biochemical mapping of a ligand-binding domain within *Arabidopsis* BAM1 reveals diversified ligand recognition mechanisms of plant LRR-RKs. Plant Journal 2012;70(5) 845-854.

[55] Schachtman DP, Goodger JQD. Chemical root to shoot signaling under drought. Trends in Plant Science 2008;13(6) 281-287.

[56] Nontachaiyapoom S, Scott PT, Men AE, Kinkema M, Schenk PM, Gresshoff PM. Promoters of orthologous *Glycine max* and *Lotus japonicus* nodulation autoregulation genes interchangeably drive phloem-specific expression in transgenic plants. Molecular Plant-Microbe Interactions 2007;20(7) 769-780.

[57] Schauser L, Roussis A, Stiller J, Stougaard J. A plant regulator controlling development of symbiotic root nodules. Nature 1999; 402(6758) 191-195.

[58] Oldroyd GED, Long SR. Identification and characterization of *nodulation-signaling pathway 2*, a gene of *Medicago truncatula* involved in Nod factor signaling. Plant Physiology 2003;131(3) 1027-1032.

[59] Kaló P, Gleason C, Edwards A, Marsh J, Mitra RM, Hirsch S, Jakab J, Sims S, Long SR, Rogers J, Kiss GB, Downie JA, Oldroyd GED. Nodulation signaling in legumes requires NSP2, a member of the GRAS family of transcriptional regulators. Science 2005;308(5729) 1786-1789.

[60] Smit P, Raedts J, Portyanko V, Debelle F, Gough C, Bisseling T, Geurts R. NSP1 of the GRAS protein family is essential for rhizobial Nod factor-induced transcription. Science 2005;308(5729) 1789-1791.

[61] Soyano T, Kouchi H, Hirota A, Hayashi M. NODULE INCEPTION Directly Targets *NF-Y* Subunit Genes to Regulate Essential Processes of Root Nodule Development in *Lotus japonicus.* Plos Genetics 2013;9(3) e1003352. http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003352 (accessed 22 July 2013)

[62] Murray JD,Karas BJ, Sato S, Tabata S, Amyot L, Szczyglowski K. A cytokinin perception mutant colonized by Rhizobium in the absence of nodule organogenesis. Science 2007;315(5808) 101-104.

[63] Tirichine L, Sandal N, Madsen LH, Radutoiu S, Albrektsen AS, Sato S, Asamizu E, Tabata S, Stougaard J. A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. Science 2007;315(5808) 104-107.

[64] Heckmann AB, Sandal N, Bek AS, Madsen LH, Jurkiewicz A, Nielsen MW, Tirichine L, Stougaard J. Cytokinin induction of root nodule primordia in *Lotus japonicus* is regulated by a mechanism operating in the root cortex. Mol Plant Microbe Interactions 2011;24(11) 1385-1395.

[65] Plet J, Wasson A, Ariel F, Le Signor C, Baker D, Mathesius U, Crespi M, Frugier F. MtCRE1-dependent cytokinin signaling integrates bacterial and plant cues to coordi-

nate symbiotic nodule organogenesis in *Medicago truncatula*. Plant Journal 2011; 65(4) 622-633.

[66] Ariel F, Brault-Hernandez M, Laffont C, Huault E, Brault M, Plet J, Moison M, Blanchet S, Ichanté JL, Chabaud M, Carrere S, Crespi M, Chan RL, Frugier F. Two Direct Targets of Cytokinin Signaling Regulate Symbiotic Nodulation in *Medicago truncatula*. Plant Cell 2012;24(9) 3838-3852.

[67] Maekawa T, Maekawa-Yoshikawa M, Takeda N, Imaizumi-Anraku H, Murooka Y, Hayashi M. Gibberellin controls the nodulation signaling pathway in *Lotus japonicus*. Plant Journal 2009;58(2) 183-194.

[68] Mortier V, De Wever E, Vuylsteke M, Holsters M, Goormachtig S. Nodule numbers are governed by interaction between CLE peptides and cytokinin signaling. Plant Journal 2012;70(3) 367-376.

[69] Konishi M, Yanagisawa S. Identification of a nitrate-responsive cis-element in the *Arabidopsis NIR1* promoter defines the presence of multiple cis-regulatory elements for nitrogen response. Plant Journal 2010;63(2) 269-282.

[70] Konishi M, Yanagisawa S. *Arabidopsis* NIN-like transcription factors have a central role in nitrate signalling. Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants. Nature communications 2013;4 1617. http://www.nature.com/ncomms/journal/v4/n3/full/ncomms2621.html (accessed 22 July 2013)

[71] Marchive C, Roudier F, Castaings L, Bréhaut V, Blondet E, Colot V, Meyer C, Krapp A. Nature communications 2013;4 1713. http://www.nature.com/ncomms/journal/v4/n4/full/ncomms2650.html (accessed 22 July 2013)

[72] Schauser L, Wieloch W, Stougaard J. Evolution of NIN-like proteins in *Arabidopsis*, rice, and *Lotus japonicus*. Journal of Molecular Evolution 2005;60(2) 229-237.

[73] Yokota K, Hayashi M. Function and evolution of nodulation genes in legumes. Cellular and Molecular Life Sciences 2011;68(8):1341-1351.

[74] Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM. Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. Proceedings of the National Academy of Sciences of the United States of America 2011;108(45) 18524-18529.

[75] Puig J, Pauluzzi G, Guiderdoni E, Gantet P. Regulation of Shoot and Root Development through Mutual Signaling. Molecular Plant 2012;5(5) 974-983.

[76] Lin YH, Ferguson BJ, Kereszt A, Gresshoff PM. Suppression of hypernodulation in soybean by a leaf-extracted, NARK- and Nod factor-dependent, low molecular mass fraction. New Phytologist 2010;185(4) 1074-1086.

[77] Ferguson BJ, Mathesius U. Signaling interactions during nodule development. Journal of Plant Growth Regulation 2003;22(1) 47-72.

[78] Mathesius U. Auxin: at the root of nodule development? Functional Plant Biology 2008; 35(8) 651-668.

[79] van Noorden GE, Ross JJ, Reid JB, Rolfe BG, Mathesius U. Defective long-distance auxin transport regulation in the *Medicago truncatula* super numeric nodules mutant. Plant Physiology 2006;140(4) 1494-1506.

[80] Kinkema M, Gresshoff PM. Investigation of downstream signals of the soybean autoregulation of nodulation receptor kinase GmNARK. Molecular Plant-Microbe Interactions 2008;21(10) 1337-1348.

[81] Nakagawa T, Kawaguchi M. Shoot-applied MeJA suppresses root nodulation in *Lotus japonicus*. Plant and Cell Physiology 2006;47(1) 176-180.

[82] Seo HS, Li J, Lee SY, Yu JW, Kim KH, Lee SH, Lee IJ, Paek NC. The hypernodulating nts mutation induces jasmonate synthetic pathway in soybean leaves. Molecules and Cells 2007;24(2) 185-193.

[83] Terakado J,Fujihara S, Goto S, Kuratani R, Suzuki Y, Yoshida S, Yoneyama T. Systemic effect of a brassinosteroid on root nodule formation in soybean as revealed by the application of brassinolide and brassinazole. Soil Science and Plant Nutrition 2005;51(3) 389-395.

[84] Ferguson BJ, Ross JJ, Reid JB. Nodulation Phenotypes of Gibberellin and Brassinosteroid Mutants of Pea. Plant Physiology 2005;138(4) 2396-2405.

[85] Postma JG, Jacobsen E, Feenstra WJ. Three pea mutations with an altered nodulation studied by genetic-anlaysis and grafting. Journal of Plant Physiology 1988;132(4) 424-430.

[86] Ishikawa K, Yokota K, Li YY, Wang YX, Liu CT, Suzuki S, Aono T, Oyaizu H. Isolation of a novel root-determined hypernodulation mutant *rdh1* of *Lotus japonicus.* Soil Science and Plant Nutrition 2008;54(2) 259-263.

[87] Magori S, Oka-Kira E, Shibata S, Umehara Y, Kouchi H, Hase Y, Tanaka A, Sato S, Tabata S, Kawaguchi M. *Too much love*, a root regulator associated with the long-distance control of nodulation in *Lotus japonicus*. Molecular Plant-Microbe Interactions 2009;22(3) 259-268.

[88] Yoshida C, Funayama-Noguchi S, Kawaguchi M. *plenty*, a novel hypernodulation mutant in *Lotus japonicus.* Plant and Cell Physiology 2010;51(9) 1425-1435.

[89] Schnabel EL, Kassaw TK, Smith LS, Marsh JF, Oldroyd GE, Long SR, Frugoli JA. The *ROOT DETERMINED NODULATION1* gene regulates nodule number in roots of *Medicago truncatula* and defines a highly conserved, uncharacterized plant gene family. Plant Physiology 2011;157(1) 328-340.

[90] Penmetsa RV, Uribe P, Anderson J, Lichtenzveig J, Gish JC, Nam YW, Engstrom E, Xu K, Sckisel G, Pereira M, Baek JM, Lopez-Meyer M, Long SR, Harrison MJ, Singh KB, Kiss GB, Cook DR. The *Medicago truncatula* ortholog of *Arabidopsis* EIN2, sickle, is a negative regulator of symbiotic and pathogenic microbial associations. Plant Journal 2008;55(4) 580-595.

[91] Penmetsa RV, Cook DR. A Legume Ethylene-Insensitive Mutant Hyperinfected by Its Rhizobial Symbiont. Science 1997;275(5299) 527-530.

[92] Prayitno J, Rolfe BG, Mathesius U. The Ethylene-insensitive sickle mutant of *Medicago truncatula* shows altered auxin transport regulation during nodulation. Plant Physiology 2006;142(1) 168-180.

[93] Novak K. Early action of pea symbiotic gene *NOD3* is confirmed by adventitious root phenotype. Plant Science 2010;179(5) 472-478.

[94] Li DX, Kinkema M, Gresshoff PM. Autoregulation of nodulation (AON) in *Pisum sativum* (pea) involves signalling events associated with both nodule primordia development and nitrogen fixation. Journal of Plant Physiology 2009;166(9) 955-967.

[95] Takahara M, Magori S, Soyano T, Okamoto S, Yoshida C, Yano K, Sato S, Tabata S, Yamaguchi K, Shigenobu S, Takeda N, Suzaki T, Kawaguchi M. Too much love, a novel Kelch repeat-containing F-box protein, functions in the long-distance regulation of the legume-Rhizobium symbiosis. Plant and Cell Physiology 2013;54(4) 433-447.

[96] Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA. FKF1 F-BOX protein mediates cyclic degradation of a repressor of CONSTANS in *Arabidopsis*. Science 2005;309(5732) 293-297.

[97] Andriankaja A, Boisson-Demier A, Frances L, Sauviac L, Jauneau A, Barker DG, de Carvalho-Niebel F. AP2-ERF transcription factors mediate Nod factor-dependent Mt *ENOD11* activation in root hairs via a novel cis-regulatory motif. Plant Cell 2007;19(9) 2866-2885.

[98] Hirsch S, Kim J, Munoz A, Heckmann AB, Downie JA, Oldroyd GED. GRAS Proteins Form a DNA Binding Complex to Induce Gene Expression during Nodulation Signaling in *Medicago truncatula.* Plant Cell 2009;21(2) 545-557.

[99] Cerri MR, Frances L, Laloum T, Auriac MC, Niebel A, Oldroyd GED, Barker DG, Fournier J, de Carvalho-Niebel F. *Medicago truncatula* ERN Transcription Factors: Regulatory Interplay with NSP1/NSP2 GRAS Factors and Expression Dynamics throughout Rhizobial Infection. Plant Physiology 2012;160(4) 2155-2172.

[100] Murakami Y, Yokoyama H, Fukui R, Kawaguchi M. Down-Regulation of NSP2 Expression in Developmentally Young Regions of *Lotus japonicus* Roots in Response to Rhizobial Inoculation. Plant and Cell Physiology 2013;54(4) 518-527.

[101] Marsh JF, Rakocevic A, Mitra RM, Brocard L, Sun J, Eschstruth A, Long SR, Schultze M, Ratet P, Oldroyd GED. *Medicago truncatula NIN* is essential for rhizobial-inde-

pendent nodule organogenesis induced by autoactive calcium/calmodulin-dependent protein kinase. Plant Physiology 2007;144(1) 324-335.

[102] Suzuki A, Hara H, Kinoue T, Abe M, Uchiumi T, Kucho K, Higashi S, Hirsch AM, Arima S. Split-root study of autoregulation of nodulation in the model legume *Lotus japonicus*. Journal of Plant Research 2008;121(2) 245-249.

[103] Saur IML, Oakes M, Djordjevic MA, Imin, N. Crosstalk between the nodulation signaling pathway and the autoregulation of nodulation in *Medicago truncatula*. New Phytologist 2011;190(4) 865-874.

[104] Hwang I, Sheen J, Muller B. Cytokinin Signaling Networks. Annual Review of Plant Biology 2012;63 353-380.

[105] Op den Camp RH, De Mita S, Lillo A, Cao Q, Limpens E, Bisseling T, Geurts R. A Phylogenetic Strategy Based on a Legume-Specific Whole Genome Duplication Yields Symbiotic Cytokinin Type-A Response Regulators. Plant Physiology 2011;157(4) 2013-2022.

[106] Leibfried A, To JP, Busch W, Stehling S, Kehle A, Demar M, Kieber JJ, Lohmann JU. WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. Nature 2005;438(7071) 1172-1175.

[107] Yadav RK, Perales M, Gruel J, Girke T, Jönsson H, Reddy GV. WUSCHEL protein movement mediates stem cell homeostasis in the *Arabidopsis* shoot apex. Genes & Development 2011;25(19) 2025-2030.

[108] Osipova MA, Mortier V, Demchenko KN, Tsyganov VE, Tikhonovich IA, Lutova LA, Dolgikh EA, Goormachtig S. *WUSCHEL-RELATED HOMEOBOX5* Gene Expression and Interaction of CLE Peptides with Components of the Systemic Control Add Two Pieces to the Puzzle of Autoregulation of Nodulation. Plant Physiology 2012;158(3) 1329-1341.

[109] Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ. Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 1997;11(9) 1963-1971.

[110] Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B. An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 1999;99(5): 463-472.

[111] Suzaki T, Yano K, Ito M, Umehara Y, Suganuma N, Kawaguchi M. Positive and negative regulation of cortical cell division during root nodule development in *Lotus japonicus* is accompanied by auxin response. Development 2012;139(21) 3997-4006.

[112] Hirsch AM, Bhuvaneswari TV, Torrey JG, Bisseling T. Early Nodulin Genes are Induced in Alfalfa Root Outgrowths Elicited by Auxin Transport Inhibitors. Proceedings of the National Academy of Sciences of the United States of America 1989;86(4) 1244-1248.

[113] Rightmyer AP, Long SR. Pseudonodule Formation by Wild-Type and Symbiotic Mutant *Medicago truncatula* in Response to Auxin Transport Inhibitors. Molecular Plant-Microbe Interactions 2011;24(11) 1372-1384.

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